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Histone acetylation by p300 is involved in CREB-mediated transcription on chromatin

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Abstract

Signal transduction through cAMP to activate gene expression via the cAMP-responsive element (CRE) is one of the most intensively studied transcription pathways. In this pathway, transcription factor CRE-binding protein (CREB) recognizes the CRE enhancer on DNA. The CREB protein is activated via phosphorylation at serine 133 by protein kinase A and then is able to recruit coactivator CREB-binding protein (CBP) and its homologue p300. This recruitment of CBP/p300 is required for transcription activation. The mechanism for CBP/p300 to participate in this transcription process is still unclear. CBP and p300 are histone acetyltransferases (HAT) and able to associate with other HAT proteins. It has been reported that the regulation of nuclear receptor-mediated transcription initiation by p300 requires chromatin and its HAT function. The data shown here indicate that the requirements for chromatin and p300 HAT activity also apply to the activation of CREB-mediated transcription. Serine 133-phosphorylated CREB recruits p300 onto chromatin for efficient acetylation of nucleosomes. This targeted acetylation by p300 is essential to CREB-dependent transcription pathway.

Keywords: Chromatin; cAMP-responsive element binding protein; Histone acetyltransferase; p300; Transcription

1. Introduction

Adenoviral E1A-binding protein p300 and CREB-binding protein CBP are highly conserved in their primary sequences and are closely related in their functions [1–5]. Many studies have suggested that CBP/p300 is critically involved in control of development, proliferation, differentiation, and apoptosis [6,7]. These physiological functions of CBP/p300 depend on its ability to serve as a transcription coactivator [1,8]. CBP and p300 have been found to participate in many signal transduction pathways involving a wide variety of transcription factors [6,9]. It has been proposed that CBP/p300 is essential and integral for many fundamental biological events. Precisely how CBP/p300 activates transcription remains uncertain, however.

CBP and p300 have been shown to interact with the basal transcriptional factors TFIIB and TFIID, as well as with the RNA polymerase II through RNA helicase A [10–12]. These observations suggest that one function of these coactivators is to serve as transcriptional integrators or adaptors and stabilize the pre-initiation complex. CBP and p300 have also

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been found to contain histone/protein acetyltransferase (HAT) activity [13,14]. They are able to interact with other HAT proteins such as PCAF, Src-1 and ACTR [15–17]. They can also acetylate other transcription factors [18–21]. Further, p300 induced estrogen receptor-mediated transcription in vitro only on the template with chromatin structure, but not on the naked DNA [22]. This chromatin-dependent transcription event required p300 HAT function [23]. Therefore, through the activities of intrinsic and associated HATs, CBP and p300 may activate transcription through acetylating nucleosomal histones and other transcription factors. It has been shown that the recruitment of two yeast HAT complexes, SAGA and NuA4, to chromatin by activators targeted factor-bound nucleosomes for acetylation [24]. This indicates that directing HAT by a specific activator to chromatin for acetylation may cause activation of specific genes. Thus, the recruitment of the HAT proteins CBP and p300 by many other activators and components of the basal transcription machinery may not only allow them to serve as adaptors, but also to direct them for acetylation of nucleosomes or other transcription factors. The acetylation by CBP and p300 and/or their associated HATs may cause remodeling of the chromatin structure or modulate the transcription complexes on the chromatin during activation of transcription on specific genes. It has been shown that p300/CBP used both HAT-dependent and HAT-independent mechanisms during transcription coactivation [23].

One of the most intensively studied transcriptional pathways is the signal transduction through cAMP to activate genes via their enhancer (CRE) [25]. This pathway consists of the transcription factor CREB, protein kinase A (PKA), and the coactivator CBP or its homologue p300 [2,5,8,26]. There is evidence suggesting that CBP/p300 may serve as an adaptor between CREB and basal transcription machinery to stabilize the pre-initiation complex [27]. It has also been reported that CREB-mediated gene expression required CBP HAT activity in vivo [28]. It is still unclear how the HAT function of CBP/p300 participates in this transcriptional process. This work is a part of the effort to understand this question. An in vitro transcription system was used containing a Gal4-CREB fusion protein to recruit p300 onto a Gal4 site-containing transcription template and HeLa nuclear extracts to provide the basal transcription machinery. Chromatin structure had been reconstituted on this transcription template. In vitro transcription assays using this system and in vivo transfection experiments using p300 mutants revealed that acetylation of chromatin by p300 played a major role in CREB-mediated transcription.

2. Materials and methods

2.1. Preparation of short chromatin

The short chromatin was prepared from HeLa cells as described previously [29,30]. Briefly, nuclei isolated from HeLa cells were washed with BC600 (600 mM KCl in the BC buffer containing 20 mM Tris–Cl, pH 7.9, 0.2 mM EDTA, 10 mM β-mercaptoethanol, and 0.5 mM PMSF) and sonicated extensively. The sonicated nuclear solution was fractionated on a Sepharose CL-6B column in BC650 containing 0.34 M sucrose. The fractions were analyzed by electrophoresis both on 1.5% agarose gel for visualizing stripped DNA and on 15% SDS–PAGE for histones. The fractions that contain short chromatin bearing less than five nucleosomes were pooled and dialyzed in BC0.

2.2. Chromatin reconstitution and analysis

Chromatin was reconstituted on pT3G5 plasmid DNA as described [31]. A mixture of the plasmid and the donor short chromatin prepared from HeLa cells was equilibrated in BC1000. This mixture was dialyzed from BC1000 to BC2000 containing 5 M urea, and then slowly and gradually to BC600 with urea in order to transfer histone octomers from the donor short chromatin onto the plasmid. The mixture was further dialyzed intensively in BC600 and then in TE. The reconstituted chromatin plasmid DNA (1.5 ml each) was purified by centrifugation on a 20–50% sucrose gradient [30]. The purified chromatin plasmid DNA was analyzed by micrococcal nuclease digestion (4 U/ml) at room temperature for various times and separated on 1.5% TAE-agarose gel [32].

2.3. Protein expression constructs

Both Gal-CREB and its point mutant at serine 133 of the CREB portion (Gal-CREB M1) was generated by fusing Gal4 amino acids (aa) 1–94 to the C-terminus of the full-length CREB aa 1–341 and subcloning the fusion gene into pET-23a vector (Novogen) between *Nde*I and *Eco*RI sites. After expression, the fusion protein contains a 6×histidine tag at the C-terminus. cDNA for PKAα catalytic subunit was subcloned into pET 24a vector (Novogen) between *Nde*I and *Bam*HI sites.

For recombinant p300 (rp300) expression construct, the full-length human p300 cDNA was fused at the N-terminus with two copies of flag tags and subcloned into pFastBac vector (BRL Biotech) between *Bss*HII and *Hin*dIII sites.

2.4. Protein expression and purification

Gal-CREB and PKA were expressed in *Escherichia coli* BL21 DE3 strain (Novogen) as recommended by the manufacturer. The bacterial cells were resuspended in BC100 (5 ml/g) and lysed by French Press. The supernatant of the cell lysate was subject to ammonium sulfate precipitation with 25–40% saturation. The precipitate was resuspended in phosphate buffer (pH 6.8) without salt and the conductivity of the solution was adjusted to 780 μS (equivalent to 250 mM KCl). Proteins were fractionated using a P11 column (Whatman). After washing the column with the phosphate buffer containing 250 mM KCl (PS250), proteins were eluted in a gradient of PS250–1500. The fractions with conductivity of 1.2–2.2 mS, containing the Gal-CREB protein, were pooled. The fractions with lower conductivity, containing PKA, were also pooled. Both Gal-CREB and PKA were further purified using Ni^2-nitriloacetic acid (NTA)-agarose affinity chromatography (Qiagen) following the manufacturer's instructions. Gal-CREB was phosphorylated by PKA through co-expression with PKA in bacteria. Alternatively, the purified Gal-CREB protein might also be phosphorylated in vitro by purified PKA (500:2, w/w) at 30°C for 20 min in 50 mM MOPS (pH 6.8), 50 mM KCl, 2 mM $MgCl_2$, 1 mM DTT and 100 μM ATP. The serine 133 mutant Gal-CREB M1 was also co-expressed with PKA in bacteria, and purified in the same way as Gal-CREB.

The flag-tagged p300 protein (rp300) was expressed with the baculovirus expression system (BRL Biotech) in Sf9 cells following the manufacturer's instructions. The cells were washed in phosphate buffered saline and lysed in BC100/0.5% NP-40. The supernatant of the cell lysate was first separated on a P11 column. After elution in a gradient of BC100–1000, the rp300-containing fractions were pooled and incubated with M2-agarose (Kodak, 1%, v/v) at 4°C for 4 h. The beads were washed in BC300/0.1% NP-40, five times, 15 ml each. The rp300 protein was eluted at 4°C for 1 h with rotating in BC100/0.1% NP-40 containing 0.2 mg/ml flag peptide (1:1, v/v).

2.5. Electrophoresis mobility shift assay

The 420 bp *Ava*I fragment of pG5 208-10 plasmid [33], containing five copies of Gal4 sites and one copy of nucleosome positioning sequence of 5S rRNA gene, was labeled with ^{32}P by Klenow. Dinucleosomes were assembled on the labeled DNA fragment using the octomer transfer method with HeLa short chromatin as donor, and further purified by centrifugation on a 5–20% sucrose gradient [34]. The binding reaction (30 μl) contained 10 mM Tris–Cl (pH 7.5), 50 mM NaCl, 1 mM EDTA, 1 mM DTT, 5% glycerol, 1 μg poly(dI-dC), the labeled dinucleosome probe (10^5 cpm), and either Gal-CREB M1 (50 ng), P-Gal-CREB (50 ng), or rp300 (200 ng). After incubation at room temperature for 30 min, the reaction was separated on 4% polyacrylamide gel in 0.5% TBE. The gel was dried before autoradiography.

2.6. HAT assay

Cold dinucleosomes containing Gal4-binding sites were prepared as described above. The dinucleosomes (150 ng) were incubated at 30°C for 10 min with either P-Gal-CREB or Gal-CREB M1 (50 ng) in 20 mM Tris–Cl (pH 7.9), 10 mM sodium butyrate, 1 mM EDTA, 1 mM DTT, 1 mM PMSF, and 10% glycerol [30]. Then, rp300 (200 ng) was added and the mixture was incubated for 10 more minutes. The acetylation reaction was initiated by adding ^{14}C-acetyl-CoA (NEN, 8 nCi) [13,35]. After incubation at 30°C for 30 min, the reaction was stopped by adding protein-loading buffer and analyzed on a 15% SDS–polyacrylamide gel.

2.7. In vitro transcription assay

Assays were carried out as described previously [24,36,37] except using HeLa nuclear extracts instead of the purified basal transcription factors. Transcription reaction (25 μl) contained BC100 buffer, 3 mM $MgCl_2$, 150 U RNase T1, 0.5 mM ATP, 0.5 mM CTP, 25 μM UTP, 100 μM 3′-*O*-methyl GTP (Pharmacia), 1 μl [α-^{32}P]UTP (NEN, 3000 Ci/mmol, 10 mCi/ml), HeLa nuclear extracts (50 μg), DNA templates, and either P-Gal-CREB (100 ng) or rp300 (400 ng). pT3G5 plasmid, either naked DNA (100 ng) or reconstituted chromatin (1 μg), was used as a transcription template. A core promoter template (pMLΔ53, 40 ng) was also included in the reaction as control [38]. As indicated, acetyl coenzyme A (acetyl-CoA) (1 μM) or deacetylase inhibitor trichostatin A (TSA, 5 μM) may be added first with the templates. After incubation at 30°C for 60 min, the reaction was stopped by adding 200 μl stop solution (20 mM Tris–Cl, pH 8.0, 1 mM EDTA, 100 mM NaCl, and 1% SDS) and 1 μg tRNA as carrier. After extraction with phenol/chloroform (1:1), RNA was precipitated with ethanol, and analyzed on a 5% polyacrylamide gel containing 8 M urea. After electrophoresis, the gel was soaked in dH_2O, dried, and subjected to autoradiography.

2.8. Transfection and CAT assay

HeLa cells at 50% confluence in 10 cm plates were transfected with Lipofectamine (16 μl, BRL) as described by the manufacturer. One or 2 days after transfection, the cells were lysed in 100 μl buffer followed by CAT assay using the organic phase extraction procedure [8]. CAT activity was normalized against co-transfected β-gal activity [39].

3. Results

Based on transcription modulation mechanism, an in vitro transcription template (pG5HMC2AT) containing five copies of heterologous Gal4-binding sites at the upstream [38,40] was modified and used in this study (Fig. 1A). The CREB-p300 transcription system was recruited to this modified template (pT3G5) through a fusion protein Gal-CREB. Because proximal Sp1 sites exist usually at the promoters of the native genes in cooperation with the upstream CRE enhancers to activate transcription, Sp1 sites were inserted into the transcription template upstream and proximal to the TATA sequence. The Gal4 sites in the template were flanked at each side by five copies of the nucleosome positioning sequences from the 5S rRNA gene of sea urchin [33] in order to help assembly of nucleosomes on the plasmid. After chromatin reconstitution by the octomer transfer method using short chromatin prepared from HeLa cells, the chromatin template DNA pT3G5 was purified on a sucrose gradient (Fig. 1B). The

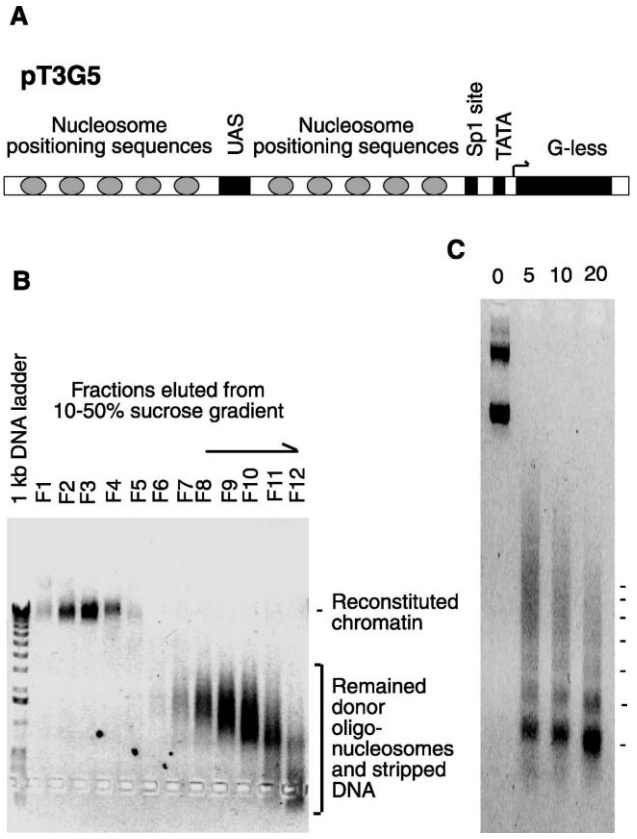

Fig. 1. Reconstitution of chromatin on transcription template. (A) Diagram of the transcription unit of the template plasmid pT3G5. UAS contains five copies of Gal4-binding sites. (B) The reconstituted chromatin was purified on sucrose gradient. The fractions eluted from the gradient were treated to strip DNA and analyzed on 1% agarose gel. The gel was stained with ethidium bromide. (C) The purified chromatin template was analyzed by micrococcal nuclease digestion followed by electrophoresis on an agarose gel. Numbers on the top represent digestion time in minutes. Dashes on the right indicate visible nucleosome arrays.

dense fractions containing pT3G5 chromatin were pooled and analyzed by digestion with micrococcal nuclease (Fig. 1C). The results of the micrococcal nuclease digestion indicate that nucleosomes were assembled on the template plasmid pT3G5 successfully.

The transcription system used in this study requires the fusion protein Gal-CREB that has been phosphorylated at serine 133 of the CREB portion by PKA. In the preparation of the phosphorylated Gal-CREB protein, Gal-CREB and PKA catalytic subunit were co-expressed in bacteria. The phosphoserine 133-specific antibody recognized the Gal-CREB purified from the co-expression bacteria (P-Gal-CREB) but not the Gal-CREB without co-expression with PKA nor the co-expressed Gal-CREB bearing a point mutation at serine 133 (Gal-CREB M1) (Fig. 2A, right). These results indicate that PKA phosphorylated Gal-CREB at serine 133 in bacteria as expected. Both purified P-Gal-CREB and Gal-CREB M1, that were co-expressed with PKA, migrated faster during electrophoresis than unphosphorylated Gal-CREB (Fig. 2A, left), suggesting that PKA might also phosphorylate CREB in bacteria at residues other than serine 133.

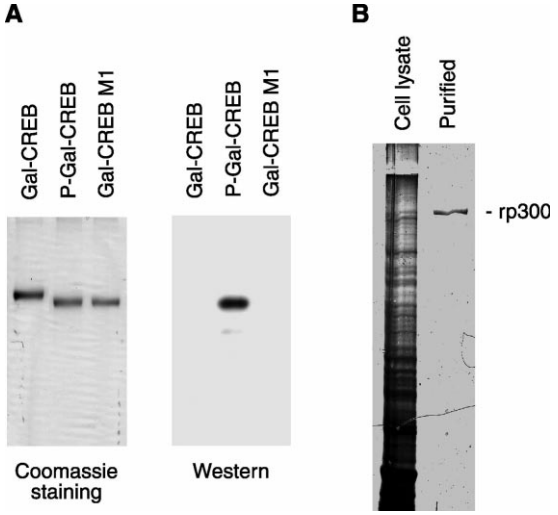

Fig. 2. Preparation of Gal-CREB and rp300 proteins. (A) Gal-CREB was expressed in *E. coli*, purified by P11 and Ni-NTA affinity chromatography, and separated on 10% SDS–PAGE. Phosphorylated Gal-CREB (P-Gal-CREB) was phosphorylated by co-expression with PKA in bacteria. Proteins were analyzed by Coomassie staining (left) and by Western blot with anti-phosphoserine 133 antibody (Upstate Biotechnology) (right). (B) Recombinant full-length p300 (rp300) was expressed in Sf9 cells and purified by P11 and M2-agarose immunochromatography. Whole cell lysate and purified rp300 were analyzed by 6% SDS–PAGE and followed by Coomassie staining.

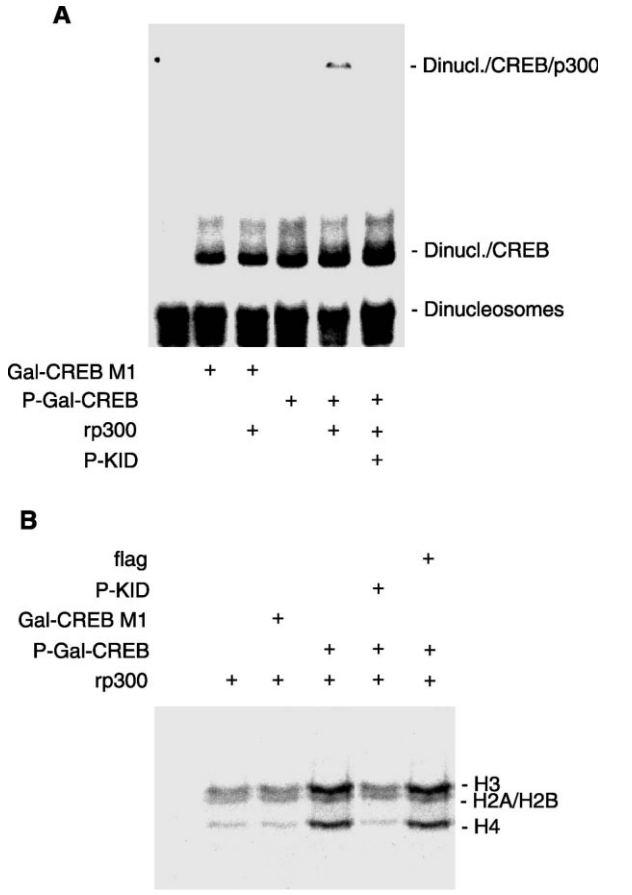

Fig. 3. Recruiting p300 by P-Gal-CREB to nucleosomes for efficient acetylation. (A) ^{32}P-Labeled and Gal4 site-containing dinucleosomes were incubated with either P-Gal-CREB or Gal-CREB M1, and then with rp300 as indicated. The binding reactions were analyzed on 4% polyacrylamide gel. Autoradiograph is shown. (B) After incubation of the cold dinucleosomes sequentially with Gal-CREB and rp300, HAT assay was followed with ^{14}C-acetyl-CoA. The labeled core histones were denatured and separated by 15% SDS–PAGE. Fluorograph is shown. The HAT reaction for each lane contained free core histones as substrates. P-KID, peptides derived from the kinase-inducible domain of CREB that are phosphorylated at serine 133; flag, the flag peptide.

To test whether the reconstituted chromatin template recruited the P-Gal-CREB and then p300 proteins normally, a ^{32}P-labeled dinucleosome probe bearing five copies of Gal4 sites and purified Gal-CREB and p300 proteins (Fig. 2A,B) were used in electrophoresis mobility shift assays. As shown in Fig. 3A, both P-Gal-CREB and its point mutant

Gal-CREB M1 bound to the dinucleosome probe. The p300 protein was recruited onto the Gal-CREB/dinucleosome complex only when the Gal-CREB had been phosphorylated, but not when the Gal-CREB M1 mutant protein was used. Thus, the recruitment of p300 onto the P-Gal-CREB/dinucleosome complex appears specific to the interaction between the KID domain of CREB containing phosphoserine 133 (P-KID) and the CREB-binding domain of p300. This result was confirmed using P-KID peptides, which blocked the formation of the p300/Gal-CREB/dinucleosome supercomplex.

Further experiments were carried out using the same dinucleosome probe but without ^{32}P labeling in order to see whether the recruitment of p300 by P-Gal-CREB to nucleosomal DNA was required for acetylation of nucleosomes. In these experiments, the Gal-CREB and p300 proteins were allowed to form complexes on the dinucleosomes first, and HAT assay was followed using ^{14}C-acetyl-CoA. Although free p300 could acetylate nucleosomes, the presence of P-Gal-CREB allowed p300 to acetylate nucleosomes more efficiently (Fig. 3B). P-KID peptides, but not irrelevant peptides (flag), repressed the acetylation in the presence of P-Gal-CREB, suggesting that the recruitment of p300 to the activator-bound nucleosomal DNA enhances acetylation of nucleosomes, i.e. previously described the HAT protein-targeted acetylation.

To answer the question of how acetylation of chromatin by p300 is involved in CREB-mediated transcription, in vitro transcription assays were performed using the template DNA pT3G5, both naked and assembled with nucleosomes. On the naked template, transcription occurred easily in the presence of the activator P-Gal-CREB (Fig. 4A). In contrast, transcription was severely repressed on the chromatin template (Fig. 4B). Similar to the previous report using the estrogen receptor transcription system [22], induction of the Gal-CREB-mediated transcription by p300 was observed only on the chromatin template but not on the naked template. There was no difference in transcription level on the naked template when P-Gal-CREB was used either with, or without, the addition of p300 (Fig. 4A). On the other hand, the transcription on the chromatin template was enhanced by the additional p300 (Fig. 4B,C). To test whether the enhancement of transcription

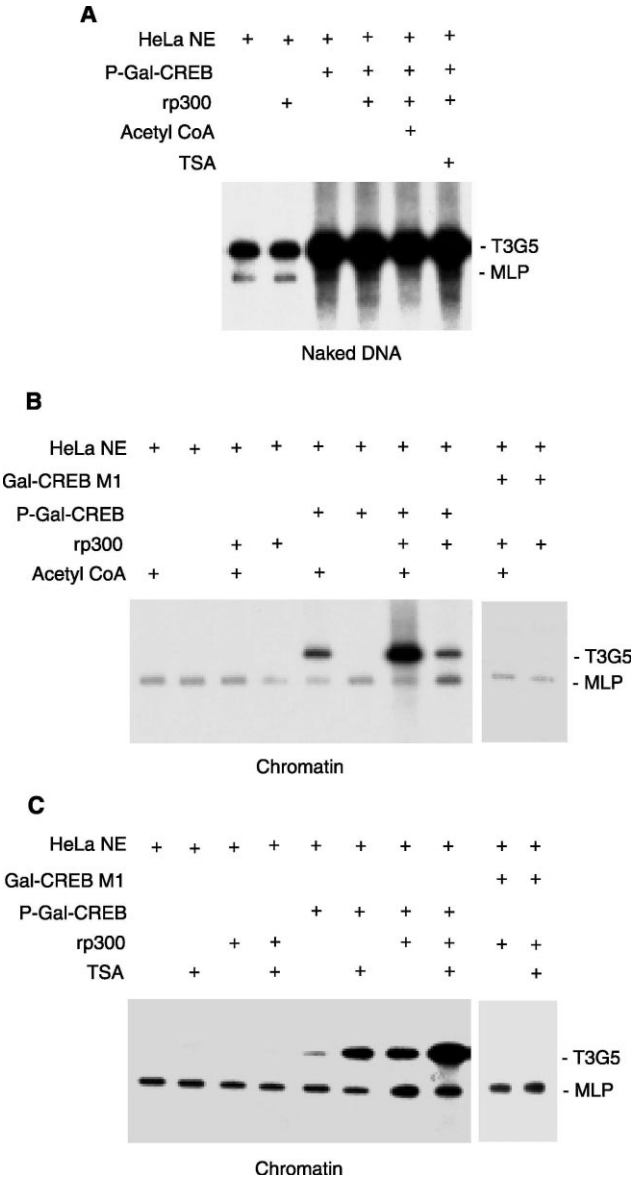

Fig. 4. Derepression of CREB-mediated transcription on chromatin by p300 through acetylation. In vitro transcription assays were carried out on either naked (A) or chromatin (B,C) template (pT3G5) with rp300 and P-Gal-CREB in HeLa nuclear extracts either in the presence or absence of acetyl-CoA or TSA. Acetyl-CoA was added in all reactions in panel C. Major late core promoter (MLP) template (pMLΔ53) was included as control in all reactions.

on the chromatin template by p300 was induced by acetylation, acetyl-CoA and the deacetylase inhibitor TSA were added into transcription reactions. Both acetyl-CoA and TSA augmented transcription on chromatin but not on naked templates (Fig. 4A–C). Acetyl-CoA may augment transcription on chroma-

tin through directly accelerating the rate of acetylation, while TSA may affect the level of acetylation by inhibiting deacetylase activities in nuclear extracts. Each reaction contained endogenous p300 provided from nuclear extracts. The improvement of CREB-mediated transcription by acetyl-CoA and TSA was observed only in the presence of P-Gal-CREB, however (Fig. 4B,C), indicating that the recruitment of p300 is required for the acetylation on chromatin. Thus, these data combined with those presented in Fig. 3 suggest that the p300 recruited onto the activator-bound chromatin template targets for efficient acetylation of nucleosomes at the promoter, therefore resulting in derepression of transcription.

We further investigated this p300-dependent transcription event in vivo using two p300 mutant expression constructs, p300 HAT− and p300 1-1737. The p300 HAT− mutant was made the same as p300mutAT2 reported previously, that lacked HAT activity [23]. The p300 1-1737 construct contains all the sequences required for HAT function but lacks CH3 and other C-terminal regions that are responsible for binding to TBP, TFIIB and other coactivators. A CRE-containing reporter and expression vectors for CREB and PKA catalytic subunit were co-transfected into HeLa cells with the expression constructs for wild type and mutant p300 constructs. The p300 proteins expressed from the transfected constructs were recovered by immunoprecipitation with anti-flag antibodies. The wild type and mutant p300 proteins were expressed at similar levels under different conditions (Fig. 5). The level of CREB in the cell was not affected by expression of either PKA or the recombinant p300 proteins. Transactivation of the CRE reporter was induced by the expression of PKA. Both the p300 HAT− and 1-1737 mutants repressed transactivation of the CRE reporter, confirming that both HAT-dependent and HAT-independent transcriptions are involved in CREB-mediated gene expression. The p300 HAT− mutant repressed the transactivation by more than 4-fold compared to the wild type while the p300 1-1737 mutant did it by less than 1-fold. These results further suggest that p300 HAT function plays a major role in CREB-dependent transcription.

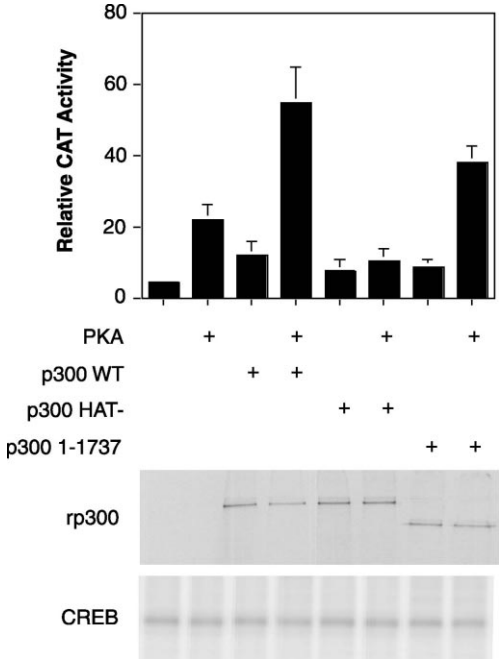

Fig. 5. Activation of CREB-mediated transcription by p300 in vivo through acetylation. HeLa cells were transfected transiently with p(−71)SRIF-CAT (1 μg) and pRc/RSV-CREB341 (1 μg). pRc/RSV-PKA (0.5 μg), and pCMV-p300 (2 μg), either wild type (WT) or mutant (HAT− or 1-1737), were co-transfected into cells as indicated. After transfection, cells were treated with TSA (400 nM) for 24 h as indicated. The upper panel shows CAT activity normalized against co-transfected β-galactosidase activity. Error bars represent standard deviation ($n=3$). The lower panels are Western analysis using either anti-p300 or anti-CREB antibodies.

4. Discussion

CBP was found, originally, to participate in cAMP-regulated transactivation of the CRE enhancer [25]. Later, its homologue, adenoviral E1A-binding protein p300, was also shown to be able to play a part in this transcription event [5]. In p300 knockout mice transactivation of the somatostatin CRE reporter was not affected, suggesting p300 itself may not be essential for this transcription pathway [41]. It is possible that both p300 and CBP can alternatively participate in the CREB-mediated transcription. This study provides further evidence that p300 can serve as a coactivator in the CREB-mediated transcription. In contrast, certain p300 functions in nuclear receptor-dependent biological events, such as activation of gene expression, upregulation of the cell cycle inhibitor p21, and induction of F9 cell differentiation, cannot be substituted by CBP [41,42]. One

explanation for this difference could be that the important role of the cAMP-induced CRE transcription in physiological control plus high demand for p300 and CBP in many biological events dictate the participation by both p300 and CBP in this CREB-mediated transcription pathway.

Although the CRE transcription pathway is one of the most intensively studied transcription events, the precise roles that both CBP and p300 play in the pathway are still unclear. The ability of CBP and p300 to interact with basal transcription factors and communicate with RNA polymerase II through RNA helicase A [10–12] suggests the possibility that these transcription coactivators may function as adaptor proteins. This idea has been challenged by the observation that stimulation of p300-induced estrogen receptor-mediated transcription required chromatin [22]. This observation is supported by the facts that p300 and CBP are HAT enzymes [13,14]. The involvement of p300 HAT function in transcription activation was observed further using the point mutant lacking HAT activity in different systems [23,28]. It has been reported that CBP/p300 coactivates transcription in both HAT-dependent and HAT-independent ways [23]. Our data using the CREB-participated transcription system support this finding and further indicate that p300 HAT function has a major contribution to this transcription process.

Our data showing p300 targeting for acetylation of nucleosomes on activator-bound nucleosomal templates during the derepression of CREB-mediated transcription on chromatin are consistent with previous detailed studies using yeast HAT complexes [24,43]. Recently, a similar study, using a similar chromatin transcription system, reported that p300-dependent transcription was enhanced by acetyl CoA and repressed by a p300 HAT-specific inhibitor [44]. The required p300 HAT function involves p300 targeting by Gal4-VP16 for histone acetylation prior to transcription. These observations are consistent with our study in that p300 targeting by Gal-CREB for acetylation of nucleosomes was involved in CREB-mediated transcription. They also showed that activators recruited p300 to chromatin templates by direct interactions, but not through the HAT domain of p300. Our study further showed that the recruitment of p300 through the specific interaction between the P-KID domain of CREB and the CREB-binding domain of p300 was required for the targeted acetylation of nucleosomes and the transcription derepression on chromatin.

In summary, p300 recruited on chromatin by PKA-phosphorylated CREB targets for acetylation of nucleosomes at the promoter, and this targeting acetylation is involved in derepression of the CREB-mediated transcription. This acetylation function of p300 plays a major role in CREB-mediated transcription activation in vivo. Further studies using purified basal transcription factors in reconstituted transcription assays, and investigating structural changes in chromatin after acetylation by p300/CBP and involvement of chromatin remodeling in p300-participated transcription derepression, will extend our understanding about the detailed mechanisms the coactivator can use in the regulation of transcription.

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